

Figure S1. Biomass $\delta^{15}\text{N}$ of *A. vinelandii* cultures grown at 0.5 μM (blue) and 5 μM (brown) Fe_T in EDTA-buffered medium. Preliminary experiments conducted under extreme Fe-limitation are shown in green. Fe_T was 0.1 μM in preliminary experiments, but Fe speciation was not controlled. Symbols denote the wild type or mutant culture: Mo-grown wild type, triangles; Mo-only mutant, squares; V-only mutant, circles. Data are plotted as a function of growth rate, with slower growth rates reflecting progressive Fe-limitation. Data points represent a single measurement of two pooled biological replicates (green) or the average of two separately measured biological replicates (blue, brown) with error bars as standard deviations. Grey shading emphasizes region where cultures using the Mo- and V-nitrogenase have similar growth rates, but distinct biomass $\delta^{15}\text{N}$.

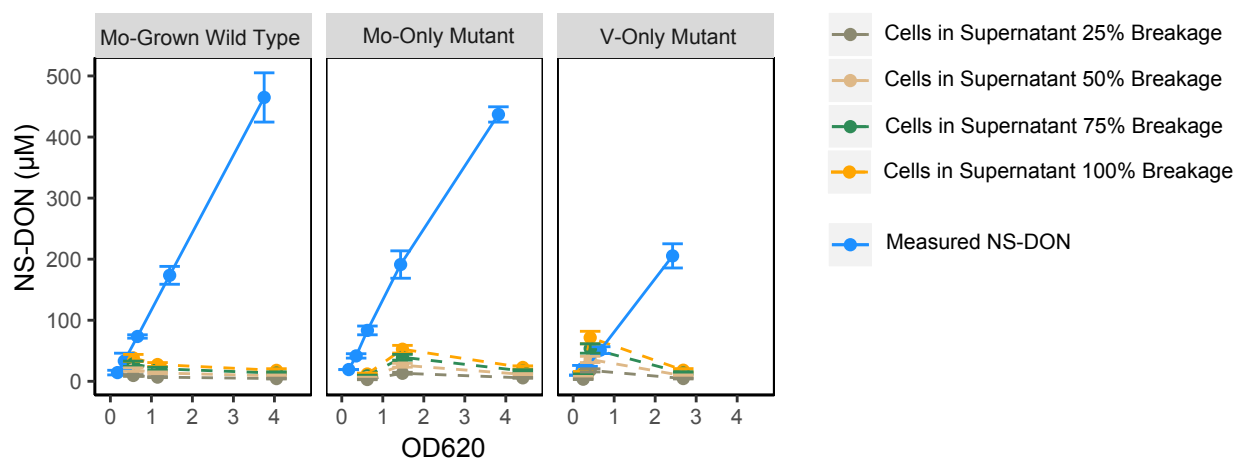


Figure S2. Estimate of potential cell lysis. Figure shows measured (non-siderophore) DON from $5 \mu\text{M Fe}_T$ experiments. Measured values are compared with estimates of potential nitrogen liberated if various percentages of cells remaining in supernatants after centrifugation were lysed. The OD620 of supernatants was measured after centrifugation, OD was converted to cell ml^{-1} and then N cell^{-1} using previously published measurements from (Bellenger et al., 2011). NS-DON: non-siderophore dissolved organic nitrogen.

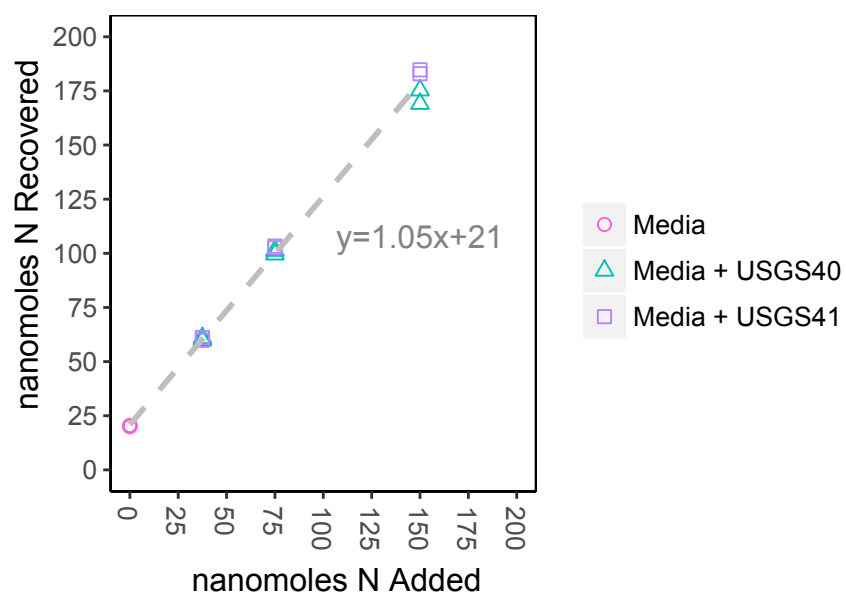


Figure S3. Oxidation of amino acid standards in *A. vinelandii* medium. Nanomoles of nitrogen recovered are plotted as a function of nanomoles USGS40 and USGS41 added. The y-intercept of 21 is consistent with the expected concentration of the chelator EDTA in the medium (100 μ M final concentration = 200 μ M N, added as 100 μ L media plus 2 mL persulfate oxidizing reagent) and suggests complete oxidation occurred.

Amino Acid	N Per Amino Acid	Treatment	Amino Acid (μM)	N (μM)
Alanine	1	Mo-Grown Wild Type	30 ± 1.59	30 ± 1.59
Arginine	4	Mo-Grown Wild Type	0.49 ± 0.05	1.97 ± 0.20
Asparagine	2	Mo-Grown Wild Type	0.11 ± 0.09	0.22 ± 0.17
Aspartic Acid	1	Mo-Grown Wild Type	nd	---
Glutamic Acid	1	Mo-Grown Wild Type	nd	---
Glutamine	2	Mo-Grown Wild Type	0.97 ± 0.21	1.9 ± 0.42
Glycine	1	Mo-Grown Wild Type	0.55 ± 0.01	0.55 ± 0.01
Histidine	3	Mo-Grown Wild Type	0.14 ± 0.20	0.4 ± 0.59
Isoleucine	1	Mo-Grown Wild Type	0.31 ± 0.01	0.31 ± 0.01
Leucine	1	Mo-Grown Wild Type	0.4 ± 0.03	0.4 ± 0.03
Lysine	2	Mo-Grown Wild Type	0.18 ± 0.00	0.35 ± 0.00
Methionine	1	Mo-Grown Wild Type	5.0 ± 0.2	5.0 ± 0.2
Phenylalanine	1	Mo-Grown Wild Type	0.34 ± 0.00	0.34 ± 0.00
Proline	1	Mo-Grown Wild Type	nd	---
Serine	1	Mo-Grown Wild Type	0.74 ± 0.06	0.74 ± 0.06
Threonine	1	Mo-Grown Wild Type	0.28 ± 0.05	0.28 ± 0.05
Tryptophan	2	Mo-Grown Wild Type	0.16 ± 0.01	0.31 ± 0.02
Tyrosine	1	Mo-Grown Wild Type	0.06 ± 0.01	0.06 ± 0.01
Valine	1	Mo-Grown Wild Type	0.36 ± 0.01	0.36 ± 0.01
Alanine	1	Mo-Only Mutant	28 ± 0.83	28 ± 0.83
Arginine	4	Mo-Only Mutant	0.53 ± 0.05	2.1 ± 0.19
Asparagine	2	Mo-Only Mutant	0.07 ± 0.00	0.13 ± 0.01
Aspartic Acid	1	Mo-Only Mutant	nd	---
Glutamic Acid	1	Mo-Only Mutant	nd	---
Glutamine	2	Mo-Only Mutant	0.79 ± 0.08	1.6 ± 0.17
Glycine	1	Mo-Only Mutant	0.48 ± 0.01	0.48 ± 0.01
Histidine	3	Mo-Only Mutant	0.59 ± 0.03	1.8 ± 0.10
Isoleucine	1	Mo-Only Mutant	0.97 ± 0.17	0.97 ± 0.17
Leucine	1	Mo-Only Mutant	1.9 ± 0.40	1.9 ± 0.40
Lysine	2	Mo-Only Mutant	0.21 ± 0.04	0.43 ± 0.08
Methionine	1	Mo-Only Mutant	7.2 ± 1.10	7.2 ± 1.10
Phenylalanine	1	Mo-Only Mutant	0.99 ± 0.21	0.99 ± 0.21
Proline	1	Mo-Only Mutant	nd	---
Serine	1	Mo-Only Mutant	0.61 ± 0.03	0.61 ± 0.03
Threonine	1	Mo-Only Mutant	0.26 ± 0.01	0.26 ± 0.01
Tryptophan	2	Mo-Only Mutant	0.28 ± 0.04	0.56 ± 0.08
Tyrosine	1	Mo-Only Mutant	0.34 ± 0.12	0.34 ± 0.12
Valine	1	Mo-Only Mutant	0.44 ± 0.01	0.44 ± 0.01
Alanine	1	V-Only Mutant	9.2 ± 1.0	9.20 ± 1.03
Arginine	4	V-Only Mutant	nd	---
Asparagine	2	V-Only Mutant	0.15 ± 0.01	0.31 ± 0.02
Aspartic Acid	1	V-Only Mutant	nd	---

Glutamic Acid	1	V-Only Mutant	nd	---
Glutamine	2	V-Only Mutant	0.58 ± 0.02	1.16 ± 0.04
Glycine	1	V-Only Mutant	0.79 ± 0.00	0.79 ± 0.00
Histidine	3	V-Only Mutant	nd	---
Isoleucine	1	V-Only Mutant	0.38 ± 0.02	0.38 ± 0.02
Leucine	1	V-Only Mutant	0.33 ± 0.00	0.33 ± 0.00
Lysine	2	V-Only Mutant	0.34 ± 0.01	0.67 ± 0.01
Methionine	1	V-Only Mutant	0.15 ± 0.00	0.15 ± 0.00
Phenylalanine	1	V-Only Mutant	0.37 ± 0.00	0.37 ± 0.00
Proline	1	V-Only Mutant	nd	---
Serine	1	V-Only Mutant	1.3 ± 0.03	1.3 ± 0.03
Threonine	1	V-Only Mutant	0.20 ± 0.01	0.20 ± 0.01
Tryptophan	2	V-Only Mutant	0.07 ± 0.05	0.14 ± 0.10
Tyrosine	1	V-Only Mutant	0.09 ± 0.00	0.09 ± 0.00
Valine	1	V-Only Mutant	0.47 ± 0.00	0.47 ± 0.00

Table S2. Amino acid content of representative *A. vinelandii* supernatants. Samples were taken from cultures grown in Fe-replete conditions (2.5 μ M Fe) after ~35 hours of growth for Mo-grown wild type and Mo-Only mutant, and ~45 hours for V-only mutant (comparable to final time points shown in main text). Cysteine was not measured. Nd: not detected. Concentrations represent the average of biological duplicates, error reported is the standard deviation. Total DON concentrations were 680 ± 77 μ M (Mo-grown wild type mutant), 780 ± 17 μ M (Mo-only mutant), 261 ± 18 μ M (V-only mutant).

Treatment	Fe _T	OD620	f_{bio} (%)	f_{DON} (%)	$\delta^{15}\text{N}_{Nfix}$ (‰)
Mo-Grown Wild-Type	0.5 μM Fe	0.6 \pm 0.01	63 \pm 0.7	37 \pm 0.7	-1.7 \pm 0.2
Mo-Grown Wild-Type	5 μM Fe	1.4 \pm 0.32	94 \pm 1.3	5.9 \pm 1.3	-1.7 \pm 0.3
Mo-Grown Wild-Type	5 μM Fe	3.8 \pm 0.03	92 \pm 0.0	8.1 \pm 0.2	-1.7 \pm 0.3
Mo-Only Mutant	0.5 μM Fe	0.6 \pm 0.02	66 \pm 1.0	34 \pm 1.0	-1.6 \pm 0.2
Mo-Only Mutant	5 μM Fe	1.4 \pm 0.37	93 \pm 1.0	6.5 \pm 1.0	-1.8 \pm 0.3
Mo-Only Mutant	5 μM Fe	3.8 \pm 0.06	92 \pm 0.2	8.0 \pm 1.4	-1.5 \pm 0.3
V-Only Mutant	0.5 μM Fe	0.5 \pm 0.01	76 \pm 0.3	24 \pm 0.3	-5.8 \pm 0.2
V-Only Mutant	5 μM Fe	2.4 \pm 0.42	96 \pm 0.4	4.3 \pm 0.4	-5.8 \pm 0.3

Table S3. Calculated $\delta^{15}\text{N}$ of Newly Fixed Nitrogen.

$\delta^{15}\text{N}_{Nfix} = \delta^{15}\text{N}_{DON} \times f_{DON} + \delta^{15}\text{N}_{bio} \times f_{bio}$, where $f_{DON} + f_{bio} = 1$. Calculations were only performed for samples with matched DON and biomass isotope measurements (Fig 3A). Propagated error for biological duplicates (\pm SD) is shown (see methods). DON: dissolved organic nitrogen, f_{bio} : fraction of nitrogen from biomass (expressed here as a percent for clarity), f_{DON} : fraction of nitrogen from DON (expressed here as a percent for clarity).

References

Bellenger, J.P., Wichard, T., Xu, Y. and Kraepiel, A.M.L. (2011) Essential metals for nitrogen fixation in a free-living N₂-fixing bacterium: chelation, homeostasis and high use efficiency. *Environmental Microbiology* 13, 1395-1411.